

## ORCHID FLORAL FRAGRANCES AND MALE EUGLOSSINE BEES: METHODS AND ADVANCES IN THE LAST SESQUIDECADE

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### ABSTRACT

All species of the Neotropical subtribes Stanhopeinae and Catasetinae (Orchidaceae) are pollinated exclusively by male euglossine bees which are attracted to and collect the floral fragrances. The orchid-euglossine bee relationship is often highly specific: the flower of a given species of plant may attract males of only one or a few species out of dozens of euglossine species in the habitat. This pollinator specificity is based upon species-specific combinations of floral fragrance compounds which attract only one or a few species of euglossine bees. Such pollinator specificity is an important reproductive isolating mechanism between sympatric interfertile species of orchids. The male bees are thought to use the collected floral fragrance compounds in their own reproductive biology, probably as precursors of their own sex pheromones.

### INTRODUCTION

One of the most striking examples of plant-insect interactions is that involving the male euglossine bees of the American tropics and the orchids they pollinate (Dodson, 1965). Although it was once thought that the male bees became “intoxicated” by the fragrances of the orchids, we now know that the situation is much different. In this paper we will review the progress that has been made since the 1969 paper on biologically active compounds in orchid floral fragrances (Dodson *et al.*, 1969). Some aspects of the biology of this group of insects were reviewed by Dressler (1982), and some aspects of the pollination biology of the orchids were reviewed by Williams (1982). Here we will emphasize the advances made in the collection and analysis of the floral fragrances, and the possible utilization of the floral fragrance compounds in the life of the insect.

Euglossine bees are exclusively Neotropical, and for the most part are solitary, communal, or quasisocial (depending on the particular species). There are three free-living genera: *Euglossa* (approximately 100 species, bright metallic blue, green, or bronze), *Eulaema* (13 species, brown or black, or striped hairy bees), and *Eufriesea* (52 species, metallic or brown/black and hairy). Two genera are nest parasites on the free-living groups: *Aglae* (monotypic, metallic blue) and *Exaerete* (5 species, metallic green). Taxonomic, biogeographic, and bibliographic references are given by Dressler (1979, 1982), Kimsey (1979, 1982), and Williams (1978, 1982).

The female bees gather food (pollen and nectar) from a variety of plants and they gather resins, mud, and other materials for nest building. The male bees visit some of the same plants as the females for food, but are not tied to the nest. The male bees leave the nest upon hatching and do not return to the nest again. They

may live a vagabond life, or they may live in one general area for extended periods of time (Ackerman *et al.*, 1982).

Orchid flowers that exhibit the "male euglossine syndrome," or "euglossine pollination" do not provide food for the visiting insect; the pollen is hidden under the anther cap, and nectar is never produced. Only male bees are attracted to the flowers, and they are attracted solely by the floral fragrances. The bees enter the flower, brush at the area where the floral fragrance is produced (using specialized brushes on the front tarsi), launch into the air and transfer the collected floral fragrance to the inflated hind tibiae. The hind tibia of the male euglossine bee is inflated and contains specialized storage and glandular tissues (Cruz-Landim *et al.*, 1965).

The orchid flowers that these bees visit to collect fragrances have only one anther, which is hidden under the anther cap. The compacted pollen masses (pollinia) are attached to a stipe (derived from the epidermis of the stigma), which in turn is attached to a viscidium (also derived from a part of the stigma). The viscidium is very sticky and is the part of the entire unit (pollinarium) which becomes attached to the insect as it leaves the flower to launch into the air. Under proper conditions one or both of the pollinia may be deposited in the stigma as a bee carrying a pollinarium leaves the flower.

The members of the Orchidaceae that attract the male bees are also found exclusively in the Neotropics. Although the most interesting pollination mechanisms are found in the orchids, the bees also visit a number of species of other families to collect the floral fragrances: *Spathiphyllum* and *Anthurium* (Araceae), *Drymonia* and *Gloxinia* (Gesneriaceae), *Cyphomandra* (Solanaceae), and *Dalechampia* (Euphorbiaceae), all of which contain one or more species that attract the male bees (Williams and Dressler, 1976; Armbruster and Webster, 1979; Dressler, 1982; Williams, 1982).

All members of the subtribes Stanhopeinae and Catasetinae (and portions of several other subtribes; see Williams, 1982, for a review) are pollinated exclusively by male euglossine bees which are attracted to and collect the floral fragrances. The orchid-euglossine bee relationship is often highly specific; the flower of a given *Stanhopea* species (for example) may attract males of only one of a few species out of dozens of euglossine species in the habitat. This pollinator specificity is based upon species-specific combinations of floral fragrance compounds which attract only one or a few species of euglossine bees. Such pollinator specificity has been shown to be an important isolating mechanism in the genus *Catasetum* (Hills *et al.*, 1972). Also, Dodson (1970) blended cineole, benzyl acetate, and alpha-pinene to match the ratio found in the fragrance of *Stanhopea tricornis*, and found that the mixture attracted only two bee species. One was *Eulaema meriana*, the known pollinator of *S. tricornis*; the other was *Euglossa dodsoni*, a bee much too small to pollinate *S. tricornis*. Thus of a set of floral visitors, only a few species may have the appropriate size or behavior to pollinate the flower successfully. Selective attraction of different pollinators can thereby act as reproductive isolating mechanisms between otherwise interfertile species. The implications concerning sympatric speciation will be discussed later in this paper.

Early work on the euglossine syndrome by Vogel (1963a, b, 1966) and Dodson and his co-workers (Dodson *et al.*, 1969) led to several suggestions of why the male bees were collecting floral fragrances. Vogel suggested that perhaps the flowers were mimicking the appearance of the nests of the female bees, but Dodson *et al.* (1969) showed that this was not a viable suggestion. Dodson *et al.* offered three tentative hypotheses to explain why the male bees collect the floral fragrances. (1) The male bees use the floral fragrances as precursors of some compounds that they cannot

normally manufacture, and thus extend their lives. This hypothesis was based on very limited data, and is now considered to be unattractive. (2) The male bees use the compounds unmodified to attract additional males of the same species to a mating site, or lek. Dodson (1975a) expanded on this hypothesis, but later studies by Kimsey (1980) do not support it. (3) Dodson *et al.* also suggested that the male bees might be using the floral fragrance compounds as precursors of a sex pheromone that would be used to attract females to a mating site. Although only a small amount of field work supports this hypothesis, it is now the favored one. In addition to being the hypothesis we favor most, it is also the one that is most complementary to the work that has been done on other groups of bees, most notably the work on bumblebees by Kullenberg and co-workers in Sweden (Kullenberg *et al.*, 1973).

Recent work on the collection of floral fragrances has centered on the use of adsorbents, although Holman has used oil impregnated glass fiber paper to collect floral fragrances. The first work reported by us on orchid floral fragrances involved the simple concentration of floral fragrances in plexiglas boxes and the direct injection of a 10 ml gas headspace sample into a gas chromatograph (Dodson and Hills, 1966; Hills *et al.*, 1968, 1972; Dodson *et al.*, 1969; Williams 1981, Williams *et al.*, 1981). This was an adequate method for the time, using  $\frac{1}{4}$  inch packed metal columns in the gas chromatograph. We were able to identify tentatively a number of compounds from the floral fragrances of a variety of species of orchids by this method in conjunction with co-injections, comparing relative retention times, and simply smelling the peaks as they eluted from the end of the gas chromatograph column via an effluent splitter. However, this method did not allow one to obtain concentrated or liquid samples for additional chemical work, and as a result the progress on the identification of a number of the compounds in the floral fragrances came to a standstill. Bergstrom (1973) and his co-workers were apparently the first to use adsorbents to study floral fragrances. They re-worked the inlet system of their gas chromatograph to accept the pre-column collection tube, and the sample was directly injected onto the gas chromatograph column. Nilsson (1978) also used physical adsorbents to collect floral fragrances into a pre-column tube that was later directly inserted into the injection port of the gas chromatograph. The disadvantage of using a precolumn tube that is inserted directly into the injection port of the chromatograph is that all of the sample is used in one injection, and therefore the sample is not available for repeated injections. In addition, this requires a modification of the injection port of the gc which may not be feasible in some circumstances, such as when an instrument is used by a number of different investigators. An additional disadvantage is that the sample is usually destroyed, so that it is not possible to isolate individual (often unknown) compounds for additional chemical analyses. Holman (Holman and Heimermann, 1973) devised a technique using oil-impregnated glass fiber papers to collect floral fragrances. An advantage of his method is that the glass paper strips are easily mailed anywhere for field work, and no pumping mechanism is necessary for collecting the floral fragrances. There are, however, several disadvantages to his method. The method requires a reasonably elaborate preparation of the paper strips, it takes a long time to collect adequate amounts of the floral fragrance for analysis, and it was necessary to modify the injection port of the gas chromatograph.

#### ANALYTICAL METHODS

We have recently developed a method that is a modification of the precolumn tube to use physical adsorbents, and devised a desorption device that allows us to collect a liquid sample of the floral fragrance. This method has several advantages:



(1) ease of sample preparation; (2) the production of a liquid sample that can be stored indefinitely; (3) production of an abundant sample so that part of the sample can be used for gc/ms analyses; (4) other parts can be used for preparatory gas chromatography to obtain pure samples of unknown compounds for NMR, IR, or other analytical techniques for structural determination. Furthermore, this method has the advantage that it does not require any modification of the injection port of the gas chromatograph.

The inflorescence is placed in a collecting chamber (plexiglas boxes, glass test tubes, or culture tubes, depending on the size and shape of the inflorescence or flower) and connected to a glass two-stage cartridge in an air stream. Fragrance laden air is drawn through the box and cartridge, with the air first coming into contact with the Tenax in the cartridge. The second stage of the cartridge is filled with charcoal to adsorb these compounds which are not adsorbed on the Tenax, or which were rapidly desorbed from the Tenax. Flow rate through the system is approximately 500 ml/minute, and sampling time is 3–4 hours.

Fragrance is desorbed from the cartridge by placing the cartridge in a desorbing device. This device was made from a length of copper tubing with reduction fittings on each end. A gas-tight seal is obtained by using a perforated high temperature septum at each end of the cartridge. The copper tube is heated to 200°C via the use of thermostated heating tape wrapped around the tube. One end of the tube is connected to a source of nitrogen gas with a flow rate of 30 ml/minute. The gas carrying the desorbed fragrance exits the device through a series of reduction fittings and flows through a 30 cm long glass capillary tube (1 mm diameter). The glass capillary tube fits inside a drilled aluminum block, which is itself fitted with a copper cold finger inserted into a Dewar flask filled with liquid nitrogen. There is therefore a temperature gradient established along the aluminum block and the fragrance compounds condense inside the glass capillary tube. After fifteen minutes of desorbing, the capillary tube is removed and the condensed compounds are eluted with one milliliter of pentane (or hexane, either of which is HPLC grade). The eluted sample and solvent is stored in a Teflon-capped automatic sampling vial for later analysis. This procedure yields sufficient fragrance for several hundred gc/ms analyses, and the samples can be stored indefinitely. The cartridges are easily made and are re-usable. The disadvantage of the system is that it requires a source of air flow, either a vacuum pump or a faucet aspirator, and thus is not an ideal system for field work. Additional details and schematics are given by Williams and Whitten (1982) and Williams (1983).

In order to discuss subtle qualitative and quantitative variations between plants, it is necessary to test the reproducibility of the sampling techniques. The variation in fragrance composition between the first and second day on anthesis of a *Cata-setum viridiflavum* inflorescence is presented in Figure 1. Three replicate samples were taken each day (three adsorbent cartridges in parallel). The results indicate little variation between replicate samples and minor variation between days. Similar checks of variation between successively produced inflorescences reveal only minor quantitative differences. It is likely, however, that health of the plant and environmental conditions might affect fragrance compositions.

The floral fragrance samples are analyzed using gas chromatography/mass spectrometry. We currently use a Hewlett-Packard 5995B gc/ms system with electron impact ionization and fused silica capillary columns. Two 25 meter columns (OV-101) are inserted into the injection port. One column is routed to the mass spectrometer, and the second column is routed to a standard flame ionization detector (FID) and integrator. This arrangement allows us to obtain simultaneous mass spectra and integrated peak areas with a single injection.



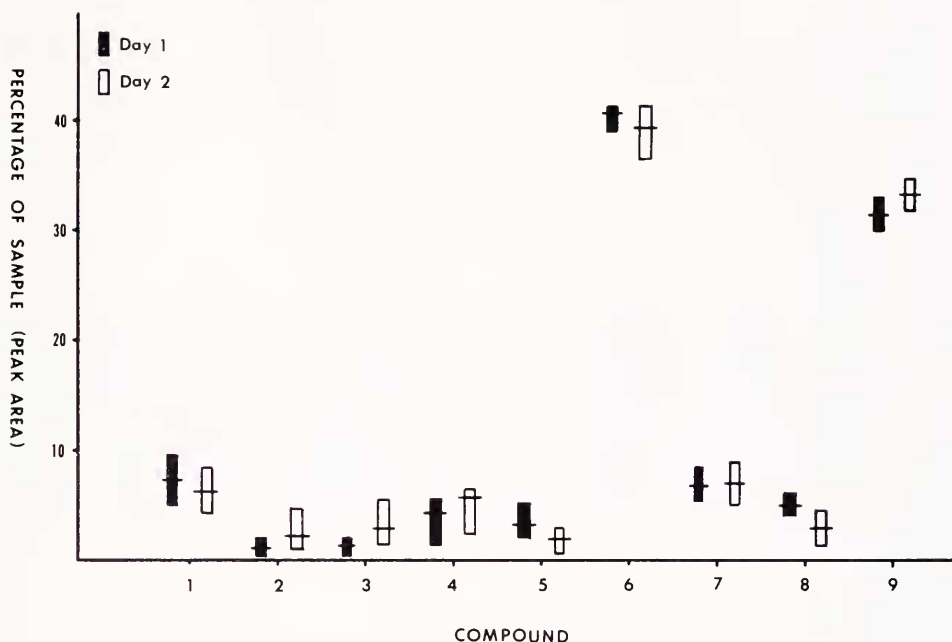


FIGURE 1. Variation in composition among replicated floral fragrance samples of a single inflorescence of *Catasetum viridiflavum*. The plant was sampled on the first and second day of anthesis, using three replicate samples (three adsorbent cartridges in parallel) per day. Bars denote the range in percent composition for each compound; horizontal marks indicate the mean.

Relevant gc/ms conditions are: helium carrier gas flow rate 1 ml/min; oven temperature programmed from 56°C to 280°C at 15°/min,  $T_1 = 2$  min,  $T_2 = 26$  min; wide bore 25 m fused silica OV-101 columns; spitless injection; injection port 300°C; transfer line 280°C; analyzer 180°C; source 150°C; FID 350°C; EM voltage 1400 V; open split interface between the column and source.

Unknown peaks of special interest can be isolated and purified via preparative gc using  $\frac{1}{4}$  or  $\frac{1}{8}$  inch packed columns (OV-101 or Carbowax 20M) connected to an effluent splitter. The splitter diverts 90% of the eluting peak to an exit port where it can be collected (either with a chilled capillary or a short trap filled with Tenax). By trapping the fragrance of a number of inflorescences, it is possible to purify several milligrams of a given fragrance compound, which is sufficient for NMR, IR, and microchemical analyses and microchemical reactions (such as ozonolysis).

## RESULTS

A number of chemicals attract male euglossine bees when presented in pure form in field trials. Many of these compounds also occur in orchid floral fragrances. Field tests of chemicals consist of simply tacking a 5 × 5 cm blotter pad to a tree or post in a forested habitat and saturating the pad with the compound to be tested. All bees attracted to the pad are collected for identification.

Table I contains those compounds identified in orchid floral fragrances, or compounds which are known to attract male euglossine bees. Most of the latter were discovered to be attractants by simply field testing large numbers of fragrant compounds. These two sets are not necessarily mutually inclusive for several reasons. First, the number of orchid species sampled is small, and new compounds will be

TABLE I

Compounds presently known to attract male euglossine bees and/or which are known to occur in fragrances of orchids pollinated by male euglossine bees

Compound	Structure	Evidence <sup>a</sup>	Attractiveness <sup>b</sup> to male bees	Plant occurrence	Abundance <sup>c</sup>
anethole MW = 148.21			*	Not yet confirmed in fragrances.	
anisaldehyde MW = 136.15			*	Not yet confirmed in fragrances.	
anisl acetate MW = 180.21 (p-methoxybenzyl acetate)		MS, RT-std	*	<i>Catasetum viridiflavum</i> <i>Gongora tricolor</i> <i>Spathiphyllum floribundum</i> (Araceae)	+ + ++
benzaldehyde MW = 106.12		MS, RT-std	*	<i>Aspasia principissa</i> <i>Catasetum viridiflavum</i> <i>Gongora bufoia</i> <i>Gongora truncata</i> <i>Spathiphyllum floribundum</i> <i>Stanhopea annulata</i> <i>Stanhopea impressa</i> <i>Stanhopea panamensis</i> <i>Stanhopea ruckeri</i> <i>Stanhopea wardii</i> <i>Trichopilia suavis</i>	+ + + + + + + + + +





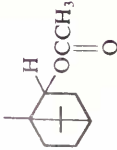

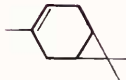
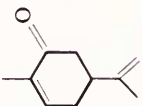
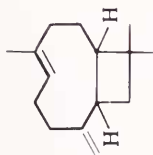
		MS, RT-std	**		
benzyl acetate MW = 150.12					
				<i>Aspasia principissa</i>	+
				<i>Aspasia variegata</i>	+
				<i>Catasetum expansum</i>	++
				<i>Catasetum longifolium</i>	++
				<i>Catasetum viridiflavum</i>	++
				<i>Clowesia thylactochila</i>	+
				<i>Gongora quinquenervis</i>	++
				<i>Spathiphyllum floribundum</i>	+++
				<i>Stanhopea annulata</i>	++
				<i>Stanhopea costaricensis</i>	+
				<i>Stanhopea impressa</i>	+
				<i>Stanhopea panamensis</i>	+
				<i>Stanhopea pulla</i>	+++
				<i>Stanhopea ruckeri</i>	+
				<i>Stanhopea tigrina</i>	+
				<i>Stanhopea wardii</i>	+
benzyl alcohol MW = 108.14		MS, RT-std	*	<i>Catasetum expansum</i>	++
				<i>Catasetum viridiflavum</i>	+
				<i>Stanhopea annulata</i>	+
				<i>Stanhopea impressa</i>	++
benzyl benzoate MW = 212.25		MS, RT-std	*	<i>Aspasia principissa</i>	+
				<i>Catasetum expansum</i>	+
				<i>Catasetum longifolium</i>	++
				<i>Catasetum viridiflavum</i>	+
				<i>Clowesia russelliana</i>	+
				<i>Cyrtanthes sp.</i>	++
				<i>Gongora cassidea</i>	+
				<i>Gongora quinquenervis</i>	+
				<i>Stanhopea panamensis</i>	+++
				<i>Stanhopea pulla</i>	+++
benzyl formate MW = 136.15			*		+
				Not yet confirmed in fragrances.	



TABLE I (Continued)

Compound	Structure	Evidence <sup>a</sup>	Attractiveness <sup>b</sup> to male bees	Plant occurrence	Abundance <sup>c</sup>
bornyl acetate MW = 196.29			*	Not yet confirmed in fragrances.	
camphene MW = 136.24		MS, RT-std	—	<i>Aspidistra variegata</i> <i>Catsetum expansum</i> <i>Catsetum macroglossum</i> <i>Catsetum maculatum</i> <i>Catsetum viridiflavum</i> <i>Clowesia russelliana</i> <i>Gongora cassidea</i> <i>Gongora quinquerivis</i> <i>Stanhopea costaricensis</i> <i>Stanhopea ecomuta</i> <i>Stanhopea gibbosa</i> <i>Stanhopea pulla</i> <i>Stanhopea wardii</i> <i>Vanilla pompona</i>	+ + + + + + +, + + + + + + +
$\Delta^3$ -carene MW = 136.24		MS, RT-std	—?	<i>Mormodes hookeri</i>	+
carvone MW = 150.22		MS, RT-std	*	<i>Catsetum expansum</i> <i>Catsetum longifolium</i> <i>Catsetum viridiflavum</i>	++ ++ ++, ++

$\beta$ -caryophyllene  
MW = 204.36



MS, RT-std

—

*Gongora armeniacae*  
*Gongora quinquenervis*  
*Gongora tricolor*

++  
+  
++

1,8-cineole  
MW = 154.25



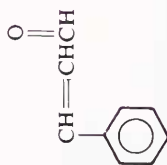
MS, RT-std

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*Aspasia variegata*  
*Clowesia russelliana*  
*Embreea rodrigasiana*  
*Gongora aceras*  
*Gongora atropurpurea*  
*Gongora cassidea*  
*Gongora gibba*  
*Gongora quinquenervis*  
*Stanhopea annulata*  
*Stanhopea candida*  
*Stanhopea costaricensis*  
*Stanhopea ecornuta*  
*Stanhopea gibbosa*  
*Stanhopea impressa*  
*Stanhopea oculata*  
*Stanhopea pulla*  
*Stanhopea ruckeri*  
*Stanhopea wardii*  
*Vanilla pompona*

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cinnamic aldehyde  
MW = 132.16



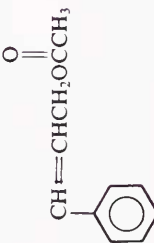
MS, RT-std

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*Clowesia thylactiochila*

+

cinnamyl acetate  
MW = 176.22




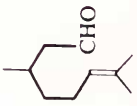
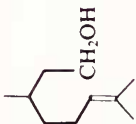

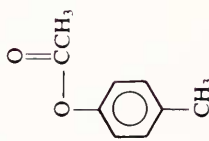
MS, RT-std

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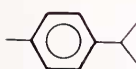
*Clowesia thylactiochila*  
*Stanhopea tigrina*  
*Zygopetalum mackayi*

++ (trans)  
+ (cis)  
++ (trans)  
+ (trans)

TABLE I (Continued)

Compound	Structure	Evidence <sup>a</sup>	Attractiveness <sup>b</sup> to male bees	Plant occurrence	Abundance <sup>c</sup>
cinnamyl alcohol MW = 134.18	<chem>CH=CHCH2OH</chem> 	MS, RT-std	*	<i>Clowesia thylacichila</i>	+
citronellal MW = 154.25		MS, RT-std	*	Not known in species that are pollinated by male bees, but is found in species of <i>Brassavola</i> .	
citronellol MW = 156.27		MS, RT-std	*	Not known in species that are pollinated by male bees, but is found in species of <i>Brassavola</i> .	
p-cresol MW = 108.14		MS, RT-std	**	<i>Gongora tricolor</i>	++
p-cresyl acetate MW = 150.18			**	Not yet confirmed in fragrances.	





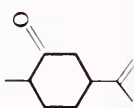
p-cymene  
MW = 134.22

MS, RT-std

—

<i>Aspidia variegata</i>	+	+
<i>Catasetum expansum</i>	+	+
<i>Catasetum integerrimum</i>	+	+
<i>Catasetum longifolium</i>	+	+
<i>Catasetum macroglossum</i>	+	+
<i>Catasetum maculatum</i>	+	+
<i>Catasetum viridiflavum</i>	+	+
<i>Clowesia russelliana</i>	+	+
<i>Clowesia warczewitzii</i>	+	+
<i>Gongora aceras</i>	+	+
<i>Gongora bufo</i>	+	+
<i>Gongora cassidea</i>	+	+
<i>Gongora gibba</i>	+	+
<i>Gongora quinquevallis</i>	+	+
<i>Gongora tricolor</i>	+	+
<i>Gongora truncata</i>	+	+
<i>Gongora unicolor</i>	+	+
<i>Mormodes sinuatum</i>	+	+
<i>Stanhopea candida</i>	+	+
<i>Stanhopea costaricensis</i>	+	+
<i>Stanhopea ecoriata</i>	0, +	+
<i>Stanhopea impressa</i>	+	+
<i>Stanhopea aff. impressa</i>	+	+
<i>Stanhopea oculata</i>	+	+
<i>Stanhopea panamensis</i>	+	+
<i>Stanhopea pulla</i>	+	+
<i>Stanhopea ruckeri</i>	+	+
<i>Stanhopea tigrina</i>	+	+
<i>Stanhopea wardii</i>	+	+

dihydrocarvone  
MW = 152.24

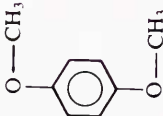


MS, RT-std

?

<i>Catasetum expansum</i>	+	+
<i>Catasetum viridiflavum</i>	+	+

p-dimethoxy benzene  
MW = 138.16

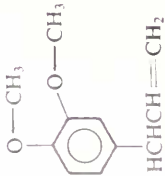
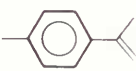
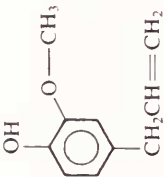
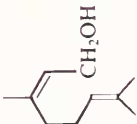
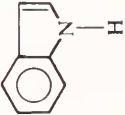


MS, RT-std

\*\*

<i>Cynoches lodigesi</i>	+	+
<i>Embreea rodigasiana</i>	+	+
<i>Gongora cassidea</i>	+	+
<i>Gongora truncata</i>	+	+
<i>Mormodes hookeri</i>	+	+
<i>Mormodes sinuatum</i>	+	+

TABLE I (Continued)

Compound	Structure	Evidence <sup>a</sup>	Attractiveness <sup>b</sup> to male bees	Plant occurrence	Abundance
1,2-dimethoxy-4(2-propenyl)benzene MW = 178.33		MS-lib	?	<i>Zygopetalum mackayi</i>	+
$\alpha$ ,p-dimethyl styrene MW = 132.21		MS, RT-std	?	<i>Catasetum expansum</i> <i>Catasetum integerrimum</i> <i>Catasetum macroglossum</i> <i>Catasetum viridiflavum</i> <i>Clowesia warezewitzii</i> <i>Gongora tricolor</i>	++ ++ ++ ++ ++ +
eugenol MW = 164.21		MS, RT-std	**	<i>Gongora aceras</i> <i>Gongora atropurpurea</i> <i>Gongora quinquerivis</i> <i>Spathiphyllum floribundum</i>	++ +++ ++ +
geraniol MW = 154.26		MS, RT-std	*	Not known in species that are pollinated by male bees, but is found in species of <i>Brassavola</i> .	
indole MW = 117.15		MS, RT-std	*	<i>Cynoches loddigesii</i> <i>Gongora cassidea</i> <i>Gongora quinquerivis</i> <i>Gongora tricolor</i> <i>Stanhopea candida</i> <i>Stanhopea aff. impressa</i> <i>Stanhopea tigrina</i>	++ ++ ++ ++ ++ ++ +

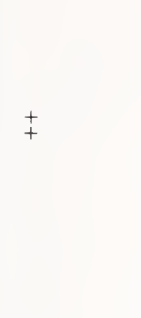
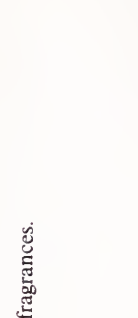


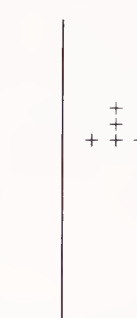
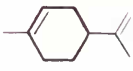
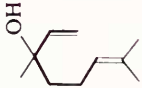
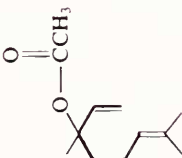
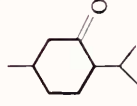
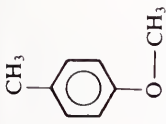
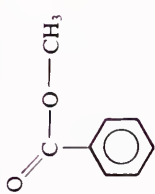
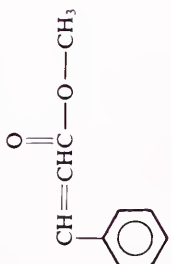
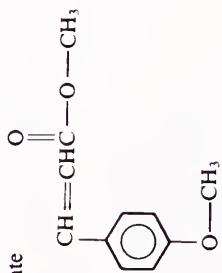
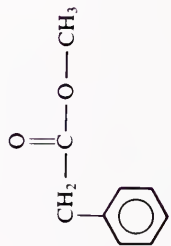
ipsdienol MW = 152.24		MS-lib	?	<i>Clowesia warczewitzii</i>	++
$\alpha$ -ionone MW = 192.30			*	Not yet confirmed in fragrances.	
$\beta$ -ionone MW = 192.30			*	Not yet confirmed in fragrances.	
$\alpha$ -irone MW = 206.33			*	Not yet confirmed in fragrances.	
isolemicin MW = 208.26		MS-lib	?	<i>Clowesia warczewitzii</i> <i>Gongora gibba</i> <i>Gongora tricolor</i>	+ ++ ++



TABLE I (Continued)

Compound	Structure	Evidence <sup>a</sup>	Attractiveness <sup>b</sup> to male bees	Plant occurrence	Abundance <sup>c</sup>
limonene MW = 136.24		MS, RT-std	*	<i>Catsetum expansum</i> <i>Catsetum integririmum</i> <i>Catsetum longifolium</i> <i>Catsetum macroglossum</i> <i>Catsetum maculatum</i> <i>Catsetum viridiflavum</i> <i>Gongora aceras</i> <i>Gongora quinquenervis</i> <i>Stanhopea panamensis</i> <i>Vanilla pompona</i>	+ + + + + + + + + +
linalool MW = 154.25		MS, RT-std	*	<i>Gongora bufoia</i> <i>Gongora cassidea</i>	+ +
linalyl acetate MW = 196.26			*	Not yet confirmed in fragrances.	
dl-menthone MW = 154.25			*	Not yet confirmed in fragrances.	

p-methyl anisole MW = 122.17		MS, RT-std	?	<i>Gongora tricolor</i>	++
methyl benzoate MW = 136.15		MS, RT-std	**	<i>Catantactum maculatum</i> <i>Cynoches</i> sp. <i>Stanhopea candida</i> <i>Stanhopea panamensis</i>	+ + + +, ++
methyl cinnamate MW = 162.19		MS, RT-std	**	<i>Aspasia epidendroides</i> <i>Embreea rodigasiana</i> <i>Gongora cassidea</i> <i>Gongora quinquenervis</i> <i>Gongora superflua</i> <i>Gongora truncata</i> <i>Stanhopea candida</i> <i>Stanhopea costaricensis</i> <i>Stanhopea ecornuta</i> <i>Stanhopea embreei</i>	+, ++ + + + ++ + + + ++ ++
methyl-p-methoxy cinnamate MW = 192.21		MS, RT-std	*	<i>Gongora quinquenervis</i>	++
methyl phenyl acetate MW = 150.18		MS, RT-std	*	<i>Gongora quinquenervis</i>	+





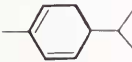
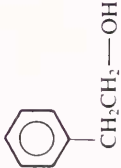



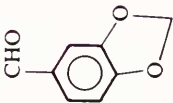
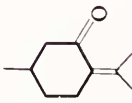
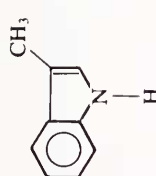
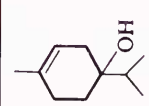
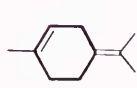
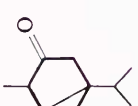
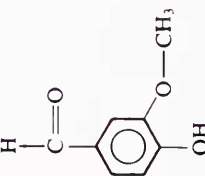
$\alpha$ -phellandrene MW = 136.24		MS, RT-std	*	<i>Catasetum expansum</i> <i>Catasetum viridiflavum</i>	+ +
2-phenylethyl acetate MW = 164.21		MS, RT-std	**	<i>Catasetum viridiflavum</i> <i>Clowesia russelliana</i> <i>Gongora aceras</i> <i>Gongora armeniacae</i> <i>Gongora bufo</i> <i>Gongora tricolor</i> <i>Gongora truncata</i> <i>Stanhoepia annulata</i> <i>Stanhoepia costaricensis</i> <i>Stanhoepia impressa</i> <i>Stanhoepia oculata</i> <i>Stanhoepia panamensis</i> <i>Stanhoepia tigrina</i> <i>Stanhoepia wardii</i> <i>Trichopilia suavis</i>	+ + + + + + + + + + + + + + +
2-phenylethyl alcohol MW = 122.17		MS, RT-std	**	<i>Catasetum viridiflavum</i> <i>Clowesia russelliana</i> <i>Gongora aceras</i> <i>Gongora bufo</i> <i>Gongora quinquevallis</i> <i>Gongora tricolor</i> <i>Stanhoepia annulata</i> <i>Stanhoepia costaricensis</i> <i>Stanhoepia impressa</i> <i>Stanhoepia tigrina</i> <i>Stanhoepia wardii</i>	+ + + + + + + + + + +
phenylpropyl acetate MW = 178.23		MS-lib	?	<i>Clowesia thylactochila</i>	+ 0,+,+,+ + + +

TABLE I (Continued)

Compound	Structure	Evidence <sup>a</sup>	Attractiveness <sup>b</sup> to male bees	Plant occurrence	Abundance <sup>c</sup>
$\alpha$ -pinene MW = 136.24		MS, RT-std	** [only (-) isomer is an attractant]	<i>Aspasia variegata</i> <i>Catasetum expansum</i> <i>Catasetum integerrimum</i> <i>Catasetum longifolium</i> <i>Catasetum macroglossum</i> <i>Catasetum maculatum</i> <i>Catasetum viridiflavum</i> <i>Clowesia russelliana</i> <i>Gongora aceras</i> <i>Gongora quinquerivis</i> <i>Stanthopea costaricensis</i> <i>Stanthopea ecornuta</i> <i>Stanthopea gibbosa</i> <i>Stanthopea pulla</i> <i>Stanthopea wardii</i>	+ + + + + + + + + + + + + +
$\beta$ -pinene MW = 136.24		MS, RT-std	—	<i>Catasetum viridiflavum</i> <i>Gongora quinquerivis</i>	+ +
piperonal MW = 150.13			*	Not yet confirmed in fragrances.	
pulegone MW = 152.24			*	Not yet confirmed in fragrances.	

skatole MW = 131.17 [3-methyl indole]		**	Not yet confirmed in fragrances.
terpinen-4-ol MW = 154.25		MS, RT-std **	<i>Gongora gibba</i> <i>Gongora quinquenervis</i> ++ ++, +++
terpinolene MW = 136.24		?	<i>Gongora quinquenervis</i> +
thujone MW = 152.24		*	Not yet confirmed in fragrances.
vanillin MW = 152.14		**	Vanillin is not known to occur in the fragrance of any orchid, although it does occur in the ripe fruits of species of <i>Vanilla</i> .

<sup>a</sup> Evidence: MS, Rt-std = match of mass spectrum and retention time with those of known standard. MS lib = match with mass spectral library only.

<sup>b</sup> Attractiveness: \* = moderate attractant; \*\* = good attractant.

<sup>c</sup> Abundance: + = minor component (0–20%); ++ = major component (21–90%); +++ = >90% of fragrance. Multiple symbols indicate intraspecific variation in fragrance composition.

added as more taxa are sampled. Also, there are many compounds in the sampled fragrances which we have not yet been able to identify and have not included in the table. Most of these appear to be sesquiterpenes and their derivatives. The number of possible isomers is large, and the minute amount of sample makes identification difficult. Second, several of the known fragrance components do not attract male bees when presented in pure form in field trials. Examples of these include p-cymene and camphene. These compounds are common in orchid fragrances, but do not attract any bees; instead they appear to modify the attractiveness of other compounds, such as cineole, resulting in the selective attraction of fewer species of bees. Some compounds (e.g. vanillin, skatole, p-cresyl acetate) are good "baits" for male bees, but have not been found in fragrances. Further sampling may reveal such compounds in fragrances. Alternatively, they may represent analogs of naturally occurring fragrance compounds and attract bees due to their similar structure. Examples are indole and p-cresol, and their respective analogs, skatole and p-cresyl acetate.

The data on attractiveness of the compounds to male bees should be regarded with some caution. This information has been accumulated over a 15-year period of baiting for bees, primarily from unpublished data of Dodson, Dressler, and ourselves and co-workers. Some of the compounds listed as poor attractants have been tested only a few times, and the chemical purity and isomeric composition of some baits was unknown. Also, many of the compounds are chiral and exist as two or more enantiomers. We have no information regarding the stereochemistry of the chiral fragrance compounds as they occur in orchids, but such information may prove to be important. We have recently baited with (+) and (−) isomers of several compounds (limonene, carvone, and alpha-pinene). In many previous tests dl-alpha-pinene has never attracted any bees. To our surprise, (−) alpha-pinene proved to be a good attractant of *Eulaema nigrata*, whereas (+) alpha-pinene and a racemic mixture attracted no bees. It appears that at least some species of euglossine bees can discriminate between stereoisomers, and a complete characterization of an orchid fragrance would ideally include the stereochemical configuration of the chiral components. Optically-active chromatographic packings have recently been introduced which allow enantiomers to be resolved, and identified by co-chromatography with known standards. The use of such packings should allow more complete characterizations of fragrances.

A number of the compounds in the table were only recently identified, and we have not yet had the opportunity to test them extensively in field trials. We suspect that many of them will prove to be good attractants, especially p-dimethoxy benzene, isoelemicin, methyl-p-methoxycinnamate, and terpinen-4-ol, since these compounds are the major components of various orchid fragrances.

### *Species specificity, variations, and distribution of floral fragrances*

We tentatively identified a number of floral fragrances and discussed their distribution in the genus *Catasetum* (subtribe Catasetinae) a decade ago (Hills *et al.*, 1972). We have also given the tentative identification and distribution of additional floral fragrance compounds in the genera *Anguloa*, *Lycaste*, *Mendoncella*, *Acineta*, *Houllettia*, *Luddemannia*, *Lycomormium*, *Paphinia*, and *Sievekingia* (Williams, Atwood, and Dodson, 1981; Williams, Whitten, and Dodson, 1983). All of this work was based on the headspace sampling technique developed at the University of Miami in the late 1960's and early 1970's. In this paper we will report on the confirmation of many of those identifications by mass spectrometry and the iden-

tification of additional compounds in some genera. We will use *Stanhopea* (a genus of about 50 species occurring throughout much of the Neotropics from northwestern Mexico to southeastern Brazil) as a detailed example of the variation and species-specificity in floral fragrance composition, and we will attempt to correlate variation in pollinators with differences in floral fragrance. Detailed information will also be given for a part of the genus *Catasetum*.

Unlike many other chemotaxonomic characters, the adaptive value of floral fragrances to male euglossine bee pollinated orchids is known; furthermore, the effects of variation in fragrance composition can (in theory) be related to differences in the pollinator sets attracted to the different fragrance forms. Floral fragrance composition should be useful in delimiting reproductively isolated groups within these taxa. Previous studies of *Stanhopea* floral fragrance (Dodson *et al.*, 1969; Dodson and Hills, 1966; Hills *et al.*, 1968) utilized direct injection of headspace samples into a gas chromatograph. These studies were successful in identifying some of the major fragrance compounds, and also demonstrated considerable interspecific variation in fragrances. In this study we have used combined gas chromatography/mass spectrometry to study variation in floral fragrance composition among 33 plants of 14 *Stanhopea* species and one species of *Embreea* previously included in *Stanhopea*.

In most *Stanhopea* species all flowers of a given inflorescence open simultaneously and persist for only two to five days before wilting. Fragrance production is strongest during the morning (about 0800 to 1300) which corresponds to the period of greatest fragrance collecting activity of the male bees. Fragrance production essentially ceases at night. All fragrance samples were collected between 0800 and 1300 hours on the first day of anthesis of each plant.

The plants used in this study were obtained from the Marie Selby Botanical Gardens (Sarasota, FL) and from the University of Florida. Collection localities and greenhouse accession numbers are given in Table II. Liquid preserved vouchers are deposited in our collection at the University of Florida and herbarium vouchers will be deposited at SEL. Plants were cultivated under uniform (as possible) greenhouse conditions for at least one year prior to sampling. Sampling techniques were described above.

The floral fragrance composition of the 33 plants are presented in Table III. Compounds comprising less than 1% of the total fragrance were not included in the table. A total of 50 compounds (>1%) was detected in the samples of 15 species. Eighteen of the compounds, including most of the major constituents, were identified on the basis of mass spectra and retention times. The data in Table III can be summarized as follows:

1. *Stanhopea panamensis* is characterized by large percentages of benzyl benzoate and methyl salicylate and/or methyl benzoate.
2. *Stanhopea wardii* is characterized by large percentages of phenylethyl acetate, phenylethyl alcohol, p-cymene, and cineole, but is quantitatively variable. One plant (#11) produces large amounts of benzyl benzoate.
3. *Stanhopea embreei* is unique in producing only methyl cinnamate.
4. *Stanhopea ruckeri* fragrance is distinctive; it is composed of benzyl benzoate, p-cymene, cineole, and myrcene. The presence of cineole and the lack of methyl salicylate distinguish it from *S. panamensis*; the absence of phenylethyl acetate distinguishes it from *S. wardii*.
5. The three samples of *Stanhopea costaricensis* differ qualitatively. Plant #16 is dominated by p-cymene, phenylethyl alcohol, and cineole; plant #17 is dominated

TABLE II

*Collection localities and greenhouse accession numbers of Stanhopea plants used in this study*

#	Species	Locality	Accession # <sup>a</sup>
1	<i>Stanhopea panamensis</i> Dodson ined.	Cerro Campana, Panama, Panama	UF-93
2	<i>Stanhopea panamensis</i> Dodson ined.	Cerro Campana, Panama, Panama	UF-25
3	<i>Stanhopea panamensis</i> Dodson ined.	unknown	UF-29
4	<i>Stanhopea panamensis</i> Dodson ined.	Cerro Campana, Panama, Panama	UF-35
5	<i>Stanhopea panamensis</i> Dodson ined.	unknown	UF-47
6	<i>Stanhopea panamensis</i> Dodson ined.	Cerro Campana, Panama, Panama	UF-80
7	<i>Stanhopea panamensis</i> Dodson ined.	Cerro Campana, Panama, Panama	UF-69
8	<i>Stanhopea panamensis</i> Dodson ined.	unknown	UF-91
9	<i>Stanhopea wardii</i> Lodd. ex Lindl.	Nicaragua	UF-26
10	<i>Stanhopea wardii</i> Lodd. ex Lindl.	Rio Chiriqui, Chiriqui, Panama	UF-39
11	<i>Stanhopea wardii</i> Lodd. ex Lindl.	Santa Clara, Chiriqui, Panama	UF-41
12	<i>Stanhopea wardii</i> Lodd. ex Lindl.	Pinola, Chiriqui, Panama	UF-58
13	<i>Stanhopea wardii</i> Lodd. ex Lindl.	unknown	SEL 48-465
14	<i>Stanhopea wardii</i> Lodd. ex Lindl.	Panama	SEL 23-75-31
15	<i>Stanhopea oculata</i> (Lodd.) Lindl.	Nicaragua	UF-48
16	<i>Stanhopea costaricensis</i> Rchb. f.	Nicaragua	UF-13
17	<i>Stanhopea costaricensis</i> Rchb. f.	Cerro Campana, Panama, Panama	UF-43
18	<i>Stanhopea costaricensis</i> Rchb. f.	Nicaragua	UF-59
19	<i>Stanhopea gibbosa</i> Rchb. f.	Nicaragua	UF-33
20	<i>Stanhopea embreei</i> Dodson	Ecuador	UF-213
21	<i>Stanhopea ruckeri</i> Lindl.	Nicaragua	UF-42
22	<i>Stanhopea ruckeri</i> Lindl.	Bocaycito, Nicaragua	UF-55
23	<i>Stanhopea impressa</i> Rolfe	Santo Domingo, Las Palmas, Ecuador	UF-36
24	<i>Stanhopea</i> aff. <i>impressa</i>	Piñas, El Oro, Ecuador	UF-40
25	<i>Stanhopea tigrina</i> Batem. ex Lindl.	unknown	SEL 23-75-41
26	<i>Stanhopea ecornuta</i> Lem.	unknown	UF-60
27	<i>Stanhopea pulla</i> Rchb. f.	Rio Iguanita, Colon, Panama	UF-65
28	<i>Stanhopea pulla</i> Rchb. f.	Rio Iguanita, Colon, Panama	UF-66
29	<i>Stanhopea annulata</i> Mansf.	Rio Chiquilpe, Ecuador	SEL 46-75-60
30	<i>Stanhopea annulata</i> Mansf.	Rio Palenque, Los Rios, Ecuador	UF-53
31	<i>Stanhopea anfracta</i> Rolfe	Moyabamba, Peru	SEL-23-75-38#2
32	<i>Stanhopea candida</i> Barb. Rodr.	unknown	SEL 79-1490
33	<i>Embreea rodigasiana</i> (Claes. ex Cogn.) Dodson (= <i>Stanhopea rodigasiana</i> Claes. ex Cogn.)	unknown	UF-94

<sup>a</sup> UF = University of Florida orchid collection (N. H. Williams); SEL = The Marie Selby Botanical Gardens, Sarasota, Florida.

by cineole and myrcene, and plant #18 produces cineole, phenylethyl alcohol, and myrcene.

6. *Stanhopea gibbosa* is dominated by cineole and myrcene, and resembles plant #17 of *S. costaricensis*.

7. *Stanhopea tigrina* is distinguished by a large percentage of phenylethyl acetate and the presence of cinnamyl acetate and indole.

8. *Stanhopea oculata* is dominated by cineole, p-cymene, and myrcene.

9. The two samples of *Stanhopea pulla* are qualitatively similar to each other, but differ in the relative amounts of p-cymene and benzyl acetate.

10. *Stanhopea annulata* is distinguished by large percentages of phenylethyl acetate, benzyl acetate, phenylethyl alcohol, and an unidentified compound (rt = 7.40).



TABLE III

*Floral fragrance composition of Stanhopea samples*

Compound and retention time		1. <i>S. panamensis</i>	2. <i>S. panamensis</i>	3. <i>S. panamensis</i>	4. <i>S. panamensis</i>	5. <i>S. panamensis</i>	6. <i>S. panamensis</i>	7. <i>S. panamensis</i>	8. <i>S. panamensis</i>	9. <i>S. wardii</i>
4.60		—	—	—	—	—	—	—	—	—
4.75	benzaldehyde	3.6	11.2	3.4	6.6	1.6	4.5	3.1	26.0	1.2
4.77	$\alpha$ -pinene	—	—	—	—	—	—	—	—	—
4.79		—	—	—	—	—	—	—	—	—
4.96	camphene	—	—	—	—	—	—	—	—	—
4.99		—	—	—	—	—	—	—	—	—
5.01		1.0	—	—	—	—	—	—	—	—
5.51	myrcene	—	—	—	—	—	—	—	1.0	—
5.93	p-cymene	5.0	—	1.6	—	2.2	7.2	—	2.3	13.9
5.97		—	4.7	—	—	—	—	—	—	—
6.04	limonene	1.0	—	—	—	—	—	1.0	—	—
6.05	1,8 cineole	—	—	—	—	—	—	—	—	5.6
6.07	benzyl alcohol	—	—	—	—	—	—	—	—	—
6.25		—	—	—	—	—	—	—	—	—
6.27		—	—	—	—	—	—	—	—	—
6.68	methyl benzoate	8.4	3.0	4.9	1.5	—	14.7	3.5	24.7	—
6.73		—	—	—	—	—	—	—	—	—
6.74		—	—	—	—	—	—	—	—	—
6.75		—	—	—	—	—	—	—	—	—
6.78		—	—	—	—	—	—	—	—	—
6.79		—	—	—	—	—	—	—	—	—
6.84		—	—	—	—	—	1.0	—	—	—
6.89		—	1.7	—	—	—	—	—	—	—
6.95	2-phenylethyl alcohol	—	—	—	—	—	—	—	—	27.2
6.97		—	—	—	—	1.0	—	—	—	—
7.33		—	—	—	—	—	—	—	—	—
7.40		—	—	—	—	—	—	—	—	—
7.44	benzyl acetate	4.5	—	—	—	—	—	—	—	—
7.49	p-dimethoxybenzene	—	—	—	—	—	—	—	—	—
7.89		—	—	—	—	—	1.5	—	—	—
7.91		—	—	—	—	—	—	—	—	—
7.94	methyl salicylate	—	40.4	2.3	64.7	39.7	—	84.9	1.5	—
8.00		3.6	—	—	—	—	—	—	—	—
8.65	2-phenylethyl acetate	4.6	—	—	—	—	—	—	—	47.1
8.73		3.1	—	—	—	—	—	—	—	—
9.00	indole	—	—	—	—	—	—	—	—	—
9.69	cinnamyl acetate (I)	—	—	—	—	—	—	—	—	—
9.85	methyl cinnamate	—	—	—	—	—	—	—	—	—
10.45	cinnamyl acetate (II)	—	—	—	—	—	—	—	—	—
11.14		—	—	—	—	—	—	—	—	—
11.73		—	—	—	—	—	—	—	—	—
12.14		—	—	—	—	—	—	—	—	—
13.82	benzyl benzoate	59.2	37.4	87.0	23.5	53.5	59.4	5.9	44.2	—
14.04		—	—	—	—	—	—	—	—	—

11. *Stanhopea anfracta* is dominated by myrcene and an unidentified compound (rt = 7.40) also present in *S. annulata*.

12. *Stanhopea candida* is distinguished by the presence of methyl salicylate and indole.

TABLE III (Continued)

	10. <i>S. wardii</i>	11. <i>S. wardii</i>	12. <i>S. wardii</i>	13. <i>S. wardii</i>	14. <i>S. wardii</i>	15. <i>S. oculata</i>	16. <i>S. costaricensis</i>	17. <i>S. costaricensis</i>	18. <i>S. costaricensis</i>	19. <i>S. gibbosa</i>	20. <i>S. embreei</i>	21. <i>S. ruckeri</i>	22. <i>S. ruckeri</i>
4.60	—	—	—	—	—	—	—	—	—	—	—	—	—
4.75	1.0	2.5	—	1.0	—	—	—	—	—	—	—	2.6	2.5
4.77	—	—	1.7	1.0	—	—	—	1.5	1.8	1.8	—	—	—
4.79	—	—	—	—	—	—	—	—	—	—	—	—	—
4.96	—	—	1.6	—	—	—	1.0	—	1.1	1.3	—	—	—
4.99	—	—	—	—	—	2.8	—	—	—	—	—	—	—
5.01	—	—	—	—	—	—	—	1.0	—	—	—	—	—
5.51	—	—	1.4	—	—	9.1	1.9	13.3	6.8	20.6	—	8.4	2.0
5.93	2.8	2.2	3.1	3.5	1.0	29.2	42.4	—	1.7	—	—	10.5	41.8
5.97	—	—	—	—	—	—	—	—	—	—	—	—	—
6.04	—	—	—	—	—	—	—	—	—	—	—	—	—
6.05	4.2	1.0	13.7	2.0	8.8	30.1	7.7	68.3	55.2	71.4	—	19.5	19.5
6.07	—	—	—	—	—	—	—	—	—	—	—	—	—
6.25	—	—	—	—	—	—	—	—	—	—	—	—	—
6.27	—	—	—	—	—	—	—	—	—	2.2	—	—	—
6.68	—	—	—	—	—	—	—	—	—	—	—	2.9	—
6.73	—	—	—	—	—	—	—	—	—	—	—	—	—
6.74	—	—	—	—	—	—	—	—	—	—	—	—	—
6.75	—	—	—	—	—	—	4.9	—	—	—	—	—	—
6.78	—	—	—	—	—	3.8	—	—	—	—	—	—	—
6.79	—	—	—	—	—	—	—	1.0	—	—	—	—	—
6.84	—	—	—	—	—	—	—	—	—	—	—	—	—
6.89	—	—	—	—	—	—	—	—	—	—	—	—	—
6.95	17.4	8.7	13.1	7.4	6.2	—	31.5	—	1.9	—	—	—	—
6.97	—	—	—	—	—	—	—	—	—	—	—	—	—
7.33	—	—	—	—	—	—	—	—	—	—	—	—	—
7.40	—	—	—	—	—	—	—	—	—	—	—	—	—
7.44	1.2	—	—	—	—	—	—	—	3.7	—	—	—	5.2
7.94	—	—	—	—	—	—	—	—	—	—	—	—	—
8.00	—	—	—	—	—	5.9	—	—	3.5	—	—	1.4	1.0
8.65	72.4	56.6	62.6	86.4	80.2	6.6	2.8	—	17.2	—	—	—	—
8.73	—	—	—	—	—	—	—	—	—	—	—	—	—
9.00	—	—	—	—	—	—	—	—	—	—	—	—	—
9.69	—	—	—	—	—	—	—	—	—	—	—	—	—
9.85	—	—	—	—	—	—	—	1.0	—	—	100.0	—	—
10.45	—	—	—	—	—	—	—	—	—	—	—	—	—
11.14	—	—	—	—	—	—	—	—	—	—	—	—	—
11.73	—	—	—	—	—	—	—	—	—	—	—	—	—
12.14	—	—	—	—	—	—	—	—	—	—	—	—	—
13.82	—	—	—	—	—	—	—	—	—	—	—	—	—
14.04	—	—	—	—	—	—	—	—	—	—	—	—	—

13. *Stanhopea impressa* and *Stanhopea* aff. *impressa* are qualitatively and quantitatively different.

14. *Embreea* (*Stanhopea*) *rodigasiana* produces large amounts of several unique, unidentified compounds.

A list of the known visitors and pollinators of the species examined in this study is presented in Table IV. The floral fragrances of *Stanhopea* attract fragrance-col-

TABLE III (Continued)

	23. <i>S. impressa</i>	24. <i>S. aff. impressa</i>	25. <i>S. tigrina</i>	26. <i>S. ecomita</i>	27. <i>S. pulla</i>	28. <i>S. pulla</i>	29. <i>S. annulata</i>	30. <i>S. annulata</i>	31. <i>S. anfracta</i>	32. <i>S. candida</i>	33. <i>Embreea rodigasiana</i>
4.60	—	—	—	—	1.0	—	—	—	—	—	—
4.75	3.4	—	—	—	—	—	—	1.7	1.1	—	—
4.77	—	—	—	1.5	3.2	1.0	—	—	—	—	—
4.79	—	1.6	—	—	—	—	—	—	—	—	—
4.96	—	—	—	1.1	4.2	—	—	—	—	—	—
4.99	—	—	—	—	—	—	—	—	—	—	—
5.01	—	—	—	—	—	—	—	—	—	—	—
5.51	13.6	5.7	—	20.5	—	—	3.8	23.3	37.0	—	—
5.93	1.3	40.5	2.0	1.8	41.5	4.2	—	—	—	1.1	—
5.97	—	—	—	—	—	—	3.7	—	9.3	—	—
6.04	—	—	—	—	—	—	—	—	—	—	—
6.05	—	37.1	—	66.2	18.1	11.1	3.2	1.2	1.0	1.0	4.9
6.07	38.7	—	—	—	—	—	3.0	—	—	—	—
6.25	—	—	—	1.6	—	—	—	—	—	—	—
6.27	—	—	—	—	1.0	—	—	—	—	—	—
6.68	—	—	—	—	—	—	—	—	—	1.5	—
6.70	—	—	—	—	—	—	—	—	—	—	—
6.73	—	—	—	—	5.3	—	—	—	—	—	—
6.74	—	—	—	—	—	—	—	—	—	—	—
6.75	—	—	—	—	—	—	—	—	—	—	—
6.78	—	—	—	—	—	—	—	—	—	—	—
6.79	—	—	—	—	—	—	—	—	—	—	—
6.84	—	—	—	—	—	—	—	—	—	—	—
6.89	—	—	—	—	—	—	—	—	—	—	—
6.95	7.0	—	6.5	—	—	—	9.4	2.5	—	—	—
6.97	—	—	—	—	—	—	—	—	—	—	—
7.33	—	—	—	—	3.0	8.7	2.2	—	1.8	—	—
7.40	—	—	—	—	—	—	22.7	26.6	40.3	—	—
7.44	1.4	—	1.0	—	17.6	68.4	3.2	8.1	—	—	—
7.49	—	—	—	—	—	—	—	—	—	—	6.5
7.89	—	—	—	—	—	—	—	—	—	—	—
7.91	—	—	—	—	—	1.5	—	—	—	—	—
7.94	—	—	—	—	—	—	—	—	—	88.0	—
8.00	—	1.1	—	—	—	—	—	—	—	—	—
8.65	27.7	—	69.4	—	—	—	35.0	36.7	—	—	—
8.73	—	—	—	—	—	—	—	—	—	—	—
9.00	—	9.9	3.4	—	—	—	—	—	—	6.3	—
9.69	—	—	1.0	—	—	—	—	—	—	—	—
9.85	—	—	—	1.0	—	—	—	—	—	1.0	4.9
10.45	—	—	15.8	—	—	—	—	—	—	—	—
11.14	—	—	—	—	—	—	—	—	—	—	2.1
11.73	—	1.4	—	—	—	—	—	—	—	—	—
12.14	—	—	—	—	—	—	—	—	—	—	52.7
13.82	—	—	—	—	—	2.0	—	—	—	—	—
14.04	—	—	—	—	—	—	—	—	—	—	15.8

lecting male euglossine bees to the flowers. It should be possible to explain differences in the visitors to different *Stanhopeas* in terms of differences in floral fragrance composition.

TABLE IV

*Known visitors and pollinators of the Stanhopea species examined in this study*

Orchid	Euglossine visitors and pollinators <sup>a</sup>	Citation <sup>b</sup>	Known chemical attractants <sup>c</sup> for bees
<i>Embreea rodigasiana</i>	unknown		
<i>Stanhopea anfracta</i>	unknown		
<i>Stanhopea annulata</i>	! <i>Euglossa grantii</i> Cheeseman	D	11
<i>Stanhopea candida</i>	! <i>Euglossa chlorosoma</i> Cockerell	D	1, 2, 4, 5, 7, 8, 12
<i>Stanhopea costaricensis</i>	p <i>Eufriesea rufocauda</i> (Kimsey)	H	1, 4
	p <i>Eufriesea schmidtiana</i> (Fries)	F	1, 5, 7, 8
	! <i>Eulaema luteola</i> Moure	E	—
	! <i>Eulaema meriana</i> (Oliver)	F	1, 2, 5, 6, 7, 8, 19
	! <i>Eulaema nigrita</i> Lepeletier	A	1, 3, 5, 8, 9, 13
	! <i>Eulaema seabrae</i> Moure	G	—
<i>Stanhopea ecornuta</i>	! <i>Eufriesea schmidtiana</i> (Fries)	F	1, 5, 7, 8
	! <i>Eulaema nigrita</i> Lepeletier	B	1, 3, 5, 8, 9, 13
	! <i>Eulaema seabrae</i> Moure	B	—
	n <i>Euglossa allosticta</i> Moure	F	1, 3, 5
	n <i>Euglossa imperialis</i> (Cockerell)	F	1, 2, 4, 7, 19
	n <i>Euglossa tridentata</i> Moure	F	1, 2, 3, 4, 5, 6, 7, 8, 19
<i>Stanhopea embreei</i>	! <i>Eulaema bomboides</i> Fries	D	5
<i>Stanhopea gibbosa</i>	! <i>Eulaema meriana</i> (Oliver)	B	1, 2, 5, 6, 7, 8, 19
<i>Stanhopea impressa</i>	! <i>Euglossa grantii</i> Cheeseman	D	11
<i>Stanhopea oculata</i>	! <i>Eufriesea caerulea</i> (Lepeletier)	E	1, 4, 11
<i>Stanhopea panamensis</i>	p <i>Eufriesea ornata</i> (Mocsary)	H	1, 2, 4, 8, 19
	? <i>Eufriesea mussitans</i> (Fabricius)	I	8
	n <i>Euglossa crassipunctata</i> Moure	E	1, 2, 3, 4, 5, 6, 7
	n <i>Euglossa cyanaspis</i> Moure	E	1, 3, 6, 8, 19
	n <i>Euglossa deceptrix</i> Moure	E	1, 3, 5
	n <i>Euglossa despecta</i> Moure	E	1, 3, 4, 5, 6, 8, 19
	n <i>Euglossa hemichlora</i> (Cockerell)	E	2, 4, 19
	n <i>Euglossa tridentata</i> Moure	E	1, 2, 3, 4, 5, 6, 7, 8, 19
<i>Stanhopea pulla</i>	! <i>Euglossa asarophora</i> Moure	H	1, 4, 5, 6, 12
<i>Stanhopea ruckeri</i>	unknown		
<i>Stanhopea tigrina</i>	! <i>Eufriesea caerulea</i> (Lepeletier)	E	1, 4, 11
	? <i>Euglossa viridissima</i> Fries	J	1, 4
<i>Stanhopea wardii</i>	p <i>Eufriesea chrysopyga</i> (Mocsary)	H	1, 5
	! <i>Eufriesea concava</i> (Fries)	C	1, 6, 7, 8, 11, 13, 15, 19
	p <i>Eufriesea rufocauda</i> (Kimsey)	H	1, 4
	! <i>Eulaema polychroma</i> (Fries)	B	1, 6, 8, 16, 17, 18

<sup>a</sup> ! = observed pollinating; p = bee captured carrying pollinaria; n = nonpollinating visitor; ? = role of visitor (pollinator or nonpollinator) uncertain.

<sup>b</sup> Literature citations for visitor data: A = Ackerman, in press; B = Dodson, 1965; C = Dodson, 1975a; D = Dodson, 1975b; E = Dodson, Dressler, and Williams, unpub.; F = Dressler, 1968; G = Dressler, 1979; H = Dressler, unpub.; I = Kimsey, 1982; J = Van der Pijl and Dodson, 1966.

<sup>c</sup> Chemical attractants for male euglossine bees (sources: Dodson, Dressler, and Williams, unpub.; Ackerman, unpub.; Kimsey, 1982.). 1—1,8 cineole; 2—methyl salicylate; 3—skatole; 4—eugenol; 5—methyl cinnamate; 6—beta-ionone; 7—benzyl acetate; 8—vanillin; 9—linalool; 10—2-phenylethyl alcohol; 11—2-phenylethyl acetate; 12—myrcene; 13—carvone; 14—menthone; 15—alpha-pinene; 16—piperonal; 17—thujone; 18—indole; 19—benzyl benzoate.

Dressler (1968) pointed out that pollination by fragrance-seeking male euglossine bees (androeglossophily) provides a situation in which sympatric speciation might occur. An individual which produces a unique fragrance might attract a different set of euglossine pollinators, thereby resulting in ethological isolation from other individuals. Possible selfing and inbreeding could lead to stabilization of that unique fragrance. Alternatively, a morphologically uniform species might radiate into different fragrance forms in different parts of its range, perhaps in response to differences in the available euglossine faunas. Subsequent intermixing of the fragrance forms could result in the sympatry of morphologically identical but ethologically isolated sibling species. Such sibling species have been documented in *Gongora* (Dodson *et al.*, 1969; Whitten and Williams, unpubl.). Geographic variation in the fragrance of *Stanhopea tricornis* was reported by Dodson *et al.* (1969). The fragrance variations among the three samples of *Stanhopea costaricensis* presented above are indicative of different fragrance forms.

Caution should be used in drawing conclusions from the available data on fragrances and pollinators for several reasons. First, we do not have both pollination and fragrance data for individual plants; until the range of variation within species is better known, it seems unwise to link pollination data from one individual with fragrance data from another. Second, data on fragrances and pollinators of *Stanhopea* are scanty and are often based on one or a few observations per species. Finally, some of the fragrance compounds possess stereoisomers (enantiomers), but their configurations have not been determined in the floral fragrances.

Whether observed variation in fragrance composition is biologically significant can only be determined by field experiments with live plants and with various mixtures of fragrance chemicals. There is no reason to assume that all components of a fragrance are critical to the attraction of pollinators. Some compounds act as primary attractants, while others modify their attraction potential, with the resultant attraction of only a few bee species (Williams and Dodson, 1972). Other compounds might have little or no effect on the attraction of pollinators and represent biochemical noise in the system.

Several of the *Stanhopea* species examined in this study are known to produce occasional natural hybrids. *Stanhopea annulata* and *S. impressa* are both pollinated by *Euglossa grantii* and rare hybrids are found along the western slopes of Ecuador and Colombia (Dodson, pers. comm.). The main isolating mechanism between the two species appears to be mechanical. The flowers of the hybrids are morphologically altered from either parent and the insect is not able to effect pollination; therefore, no genetic material is transferred from one species to the other and the integrity of each species is maintained. A secondary isolating mechanism appears to be geographical. *Stanhopea annulata* usually occurs from 100 to 600 meters in elevation, while *S. impressa* is usually found at 700–1500 meters (Dodson, 1975b). The fragrances of both species contain large amounts of phenylethyl acetate, which is the only known attractant for *Euglossa grantii*.

Hybridization also occurs between *Stanhopea ecornuta* and *S. costaricensis* in Central America. These species share three pollinators in common (*Eulaema seabrae*, *El. nigrita*, and *Eufriesea schmidtiana*). Based on the available data, cineole is the only major attractant common to both *Stanhopeas*. Cineole attracts a majority of euglossine species, but the modifier effects of other compounds combined with cineole are poorly known. The variation in fragrances among the three samples of *S. costaricensis* is surprising, and its significance is unknown. Four species of *Eulaema* and two of *Eufriesea* are reported to visit *S. costaricensis* (Table IV); perhaps this large number of potential pollinators reflects several fragrance varieties within this species. The hybrids between *S. ecornuta* and *S. costaricensis* are morpholog-



ically altered from the parental species, and the pollinators are unable to effect pollination (Dodson, pers. comm.). More detailed study is clearly needed.

*Stanhopea wardii* and *S. oculata* have fragrances which are qualitatively but not quantitatively similar; both contain p-cymene, cineole, and phenylethyl acetate. *Stanhopea wardii* is dominated by phenylethyl acetate and phenylethyl alcohol, while *S. oculata* is dominated by cineole and p-cymene. P-cymene is not known to attract any euglossine bees, although it is common in orchid fragrances, and the attraction potential of cineole/p-cymene mixtures is unknown. These two species are not known to hybridize, and their fragrances are apparently dissimilar enough to attract exclusive sets of pollinators.

The fragrance of *Stanhopea panamensis* contains large amounts of benzyl benzoate, and is the only *Stanhopea* known to attract *Eufriesea ornata*. Ackerman (1983 and pers. comm.) baited for bees extensively in central Panama using benzyl benzoate, and found that, overall, benzyl benzoate is a poor attractant of 15 species of euglossines, but that it is one of the best attractants of *Eufriesea ornata*. Benzyl benzoate appears to be the primary attractant of the pollinator of *Stanhopea panamensis*, and other chemicals in its fragrance (methyl salicylate, methyl benzoate) probably reduce the number of bee species attracted, with the resultant unique set of pollinators.

*Stanhopea embreei* is unique in producing a fragrance composed of pure methyl cinnamate (Williams and Whitten, 1982). This compound is the only known attractant of its pollinator, *Eulaema bomboides*. This bee also pollinates *Stanhopea frymirei* Dodson, but the plant species are allopatric and hence geographically isolated. The fragrance composition of the latter species is unknown.

*Stanhopea rodigasiana* was recently removed from *Stanhopea* on the basis of its distinctive floral and vegetative morphology and now forms the monotypic genus *Embreea* Dodson. The fragrance of this species contains several unique unidentified compounds not known from any other orchids, thereby supporting its separation from *Stanhopea*. Its pollinator is not known.

The fragrance of *Stanhopea impressa* (#23) is dominated by phenylethyl acetate, benzyl alcohol, and myrcene, and its pollinator, *Euglossa grantii*, is occasionally attracted to pure phenylethyl acetate. *Stanhopea* aff. *impressa* (#24) produces p-cymene, cineole, indole, and myrcene. No pollinator data are available, but such a striking difference in fragrance composition suggests that it might not be pollinated by *Euglossa grantii*. This plant was collected in southern Ecuador, outside the known range of true *Stanhopea impressa*, and differs morphologically from the latter in several details of floral structure. Dodson (pers. comm.) suggests that this plant bears only superficial resemblance to *S. impressa*, and may be more closely related to other taxa.

*Stanhopea tigrina* exists in at least two varieties which are probably adapted to different pollinators. One form occurs in northeast Mexico and is pollinated by *Eufriesea caerulea*. The channel formed by the tips of the column and epichile (apex of the labellum) is relatively wide, presumably to accommodate its large pollinator. The flowers are mottled with purple, and the fragrance is somewhat pungent due to the presence of indole. The second form, corresponding to *Stanhopea nigroviolacea* Morren. ex Beer., has flowers which are heavily blotched with dark purple, and the channel between the epichile and column is much narrower, perhaps to accommodate a smaller pollinator. The type specimen of *Euglossa viridissima* Friese was reportedly collected at flowers of *Stanhopea tigrina*, and this bee might be the pollinator of this southern form. *Euglossa viridissima* does not visit true *Stanhopea tigrina*. Unfortunately, neither pollination nor fragrance data are available for *S. nigroviolacea*.

The data presented above are generally consistent with the hypothesis that different species of *Stanhopea* usually produce distinct floral fragrances which results in the selective attraction of different species of euglossine pollinators. A detailed, functional understanding of the relationship is still not possible; given a particular fragrance composition, we cannot yet predict which euglossine species will be attracted. We lack adequate data on which bees are attracted to pure fragrance compounds and especially to mixtures of compounds.

Future studies of *Stanhopea* pollination should try to include both pollination observations and fragrance analysis for individual plants from known localities. Studies of intra- and interpopulational variation in fragrances are clearly needed, but are difficult due to the scarcity of plants in cultivation (and often in the field). Similarly, field studies of pollination are hampered by the rarity of flowering plants and by the short duration of the flowers. Perhaps the most profitable means of studying orchid/euglossine relationships will be to analyze fragrances of cultivated plants from known localities, and then prepare matching synthetic fragrance mixtures for use in field tests of attractiveness to male euglossine bees. This technique requires positive identification (and often chemical synthesis) of the major fragrance compounds, a goal still lacking for many of the orchid species we have sampled to date. Such synthetic fragrances are not a substitute for observation of pollination, but might prove useful in discovering the visitors of numerous orchids and other plants whose euglossine pollinators are currently unknown.

#### *Correction and confirmation of identifications in the Catasetinae*

The fragrances and pollinators of *Catasetum* (sensu lato) were surveyed by Hills *et al.* (1972). Although our collection of living plants from that study was largely dispersed in the intervening years, we have been attempting to reexamine the taxa treated in that paper, using improved fragrance analysis techniques. The following section presents corrections and additional data on fragrances of the Catasetinae.

The genus *Catasetum* consists of approximately 70 species found throughout the American tropics. With the recent segregation of *Clowesia* and *Dressleria* from *Catasetum* (Dodson, 1975c), the genus becomes much more homogeneous. One distinctive group within *Catasetum* is the *C. maculatum* complex, a group of at least nine species. Hills *et al.* (1972) stated that the fragrances of all members of the *maculatum* complex are essentially identical and are composed largely of alpha-pinene with small amounts of benzyl acetate, carvone, cineole, and other compounds. We have recently sampled a number of plants in the *C. maculatum* complex, and the results are diagrammed in Figure 2 a-j. Although some intraspecific and interspecific variation is evident, the fragrances within this sample of the complex are remarkably similar. Most of the samples are dominated by benzyl acetate, p-cymene, limonene, carvone, and an unknown compound (#16). Alpha-pinene appears to be a minor component, contrary to the earlier report. All members of the *C. maculatum* complex are pollinated primarily by *Eulaema meriana*, *Eulaema cingulata*, and *Eulaema polychroma*. Also included in Figure 2 are *Catasetum longifolium* and *Dalechampia spathulata*. *Catasetum longifolium* is not closely related to the *C. maculatum* complex, but it has a similar fragrance, attracts the same pollinators, and is reproductively isolated from sympatric species of the *C. maculatum* complex by placing the pollinarium on the underside of the bee's thorax rather than on the scutum. It is ecologically isolated by its restriction to *Mauritia* palms as hosts (Dodson, 1978). *Dalechampia spathulata* (Euphorbiaceae) is one of the few androeuglossophilous dicotyledons, and is pollinated by *Eulaema polychroma*, *E. cingulata*, and *E. luteola* (Armbruster and Webster, 1979). *Dalechampia*



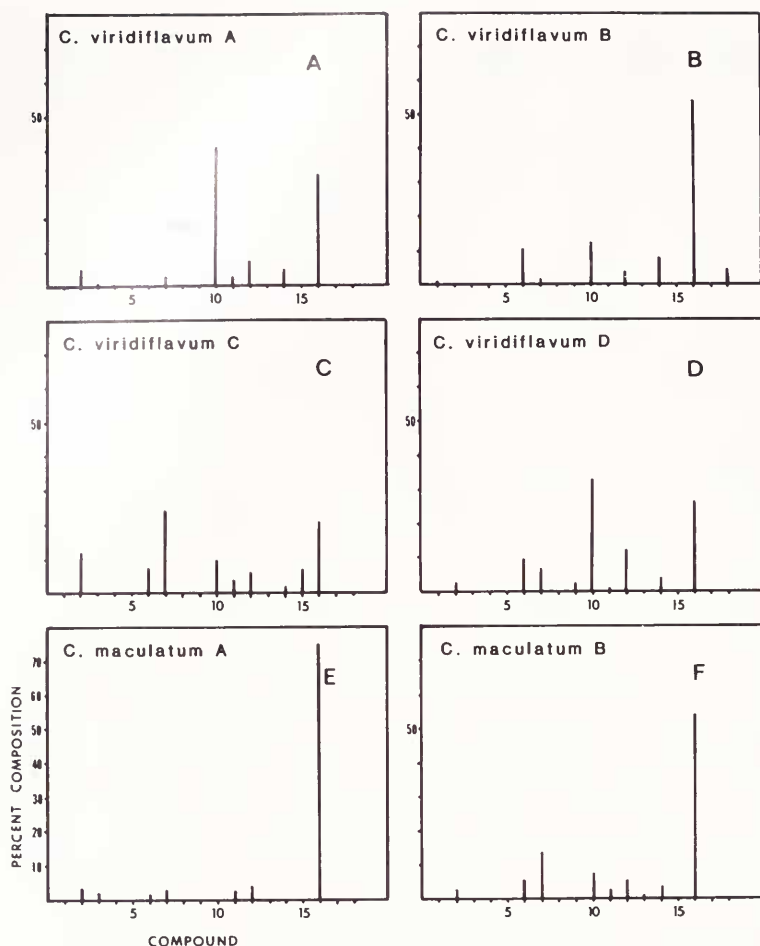


FIGURE 2. A-L. Fragrance composition of selected members of the *Catasetum maculatum* complex (A-J), *Catasetum longifolium* (K), and *Dalechampia spathulata* (L), a member of the family Euphorbiaceae. Percent composition was determined by peak areas of chromatograms. Identification of compounds: 1 = benzaldehyde; 2 =  $\alpha$ -pinene; 3 = camphene; 4 = myrcene; 5 =  $\alpha$ -phellandrene; 6 = p-cymene; 7 = limonene/cineole (only partly resolved); 8 = methyl benzoate; 9 = dimethyl styrene; 10 = benzyl acetate; 11 unidentified; 12 = dihydrocarvone; 13 unidentified; 14 = carvone; 15 = phenylethyl acetate; 16 unidentified epoxide MW = 166; 17 unidentified sesquiterpene; 18 = benzyl benzoate; 19 = methyl cinnamate.

*spathulata* and *C. longifolium* and the *C. maculatum* complex all appear to have converged upon a similar fragrance composition and hence share a common set of pollinators. Compound #16, which appears to be an epoxide related to carvone, is a major component of all the fragrances, and should prove to be a general attractant for a number of species of *Eulaema*. The other compounds present in the fragrances probably act as modifiers which restrict the numbers of *Eulaema* species attracted to the mixture. Although floral fragrances may provide characters useful in delimiting ethologically isolated sibling species of orchids, they are probably not useful in determining relationships above the species level due to the likelihood of convergence.

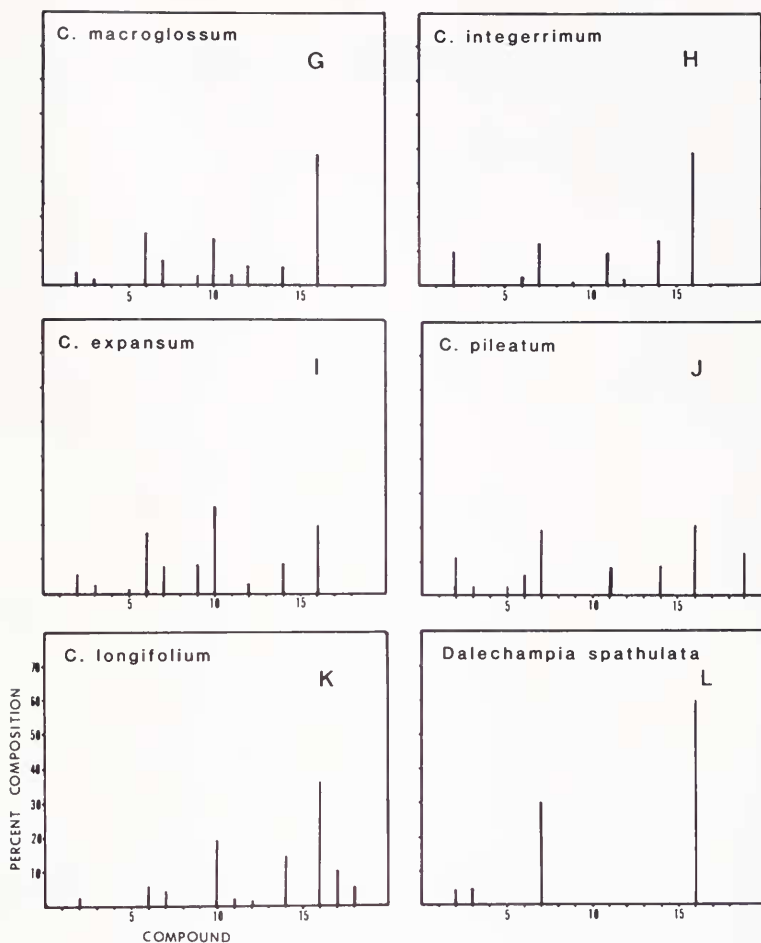


FIGURE 2 (Continued)

The genus *Clowesia* was recently segregated from *Catasetum* by Dodson (1975c). In 1972 we reported on the fragrances of four species that are now included in *Clowesia*, but which at that time were included in *Catasetum*. We have been able to obtain material of three of the four original species, and have data derived from gc/ms analyses of the fragrances of these three species. In *C. russelliana* we reported the presence of cineole as the major component of the fragrance. We have confirmed this with gc/ms. We have also confirmed the presence of alpha-pinene, and in addition we have found that the fragrance contains camphene, myrcene (which we thought lacking), p-cymene, phenylethyl alcohol (again, thought to be lacking), phenylethyl acetate (also not reported previously), and benzyl benzoate (also not reported). This is not surprising, since many of the late eluting compounds were very difficult to detect using the older headspace sampling technique.

In *Clowesia thylaciochila* we had previously reported that the major component was phenylethyl acetate; however, we are now quite sure that we were in error in our identification of this compound in the fragrance of this species. Instead, the

compound is phenylpropyl acetate, which differs from the former compound in having an additional  $\text{CH}_2$  in the side chain on the benzene ring. We have so far not confirmed the presence of phenylethyl alcohol in the fragrance of this species, and we have no reason at this time to believe that we will find this compound in the fragrance of *C. thylaciochila*. We have found benzyl acetate, cinnamaldehyde, indole, cinnamyl alcohol, and cinnamyl acetate in this fragrance also.

We had earlier suggested that because of the presence of phenyl ethyl acetate in the fragrance of *C. thylaciochila* that the probable pollinator might be *Eufriesea concava*, a species known to be strongly attracted to phenylethyl acetate. We are not able to make any predictions on the pollinator of this species, since we have not had the opportunity to test phenylpropyl acetate in field bioassays yet (this compound was identified as this paper was being written). The lack of detecting cinnamyl acetate is easily explained by the fact that this compound is a very late eluting peak on carbowax columns, which we had been using for the headspace analyses, and it is only with higher temperature, non-carbowax long capillary columns that we have been able to detect such compounds. Furthermore, such compounds are not so volatile as the faster eluting peaks, and headspace sampling is not the preferred method of sampling for this type of compound. We are eagerly awaiting the opportunity of testing phenylpropyl acetate and cinnamyl acetate in field bioassays in the very near future.

In *Clowesia warczewitzii* we have identified a number of previously unidentified compounds, and have one compound not previously found in orchid floral fragrances, ipsdienol. We confirm the presence of myrcene; p-cymene (previously unidentified); limonene; beta-ocimene (and an isomer of ocimene); alpha, p-dimethyl styrene; alpha-terpinene; terpinolene; nerol; and isoelemicin in the fragrance of *C. warczewitzii*. We suspect that the abundant unidentified compound we reported in 1972 is actually ipsdienol. We have not confirmed the presence of cineole in the fragrance of this species. The known pollinator of this plant is *Eulaema bombiformis*, which is attracted to cineole, but not to any of the compounds confirmed in the fragrance of this plant. We suspect that the bee will be attracted to some of the compounds in the fragrance but which we have not had the opportunity to subject to field assays.

*Dressleria suavis* (a segregate from *Catasetum*, Dodson 1975c) contains methyl benzoate, methyl salicylate, phenylethyl acetate, eugenol, and benzyl benzoate in its fragrance. Unfortunately, we have not been able to obtain material of the other species of *Dressleria*, and therefore have no basis of comparison with our previously reported work.

#### *Collection, storage, and utilization of floral fragrance compounds by male bees*

Our efforts to determine the fate of the fragrance chemicals collected by male euglossine bees have centered around the hypothesis that the chemicals serve as precursors for courtship or territorial pheromones. Preliminary to testing this hypothesis, we are currently analyzing the chemicals present in the hind tibial organs and the mandibular glands of as many euglossine species as possible. Preliminary work indicated that male euglossines have large mandibular glands and associated reservoirs and produce abundant secretions, while the mandibular glands of females contain very little. It seemed reasonable to suspect the mandibular gland secretions are somehow involved in sexual behavior.

Samples are obtained by collecting male bees at various fragrance baits, removing the hind tibia and the head, and extracting the parts in separate vials of hexane.

The resulting solutions are analyzed using capillary gc/ms. Such analyses show that the mandibular glands contain a variety of compounds, usually normal alkanes, alkenes, dienes, acetates, and alcohols. The composition of the head extract is highly consistent within a species, and displays great variation between species.

The hind tibia contain two sets of compounds; one set is more or less identical to the set found in the mandibular glands, and the second set consists of various fragrance compounds (mainly mono- and sesquiterpenes and aromatics). The tibial extract is often highly fragrant, reminiscent of some perfumes. Many of the compounds that occur in orchid fragrances can be found in the hind tibia of the bees that visit the orchids. This set of fragrance compounds shows considerable variation from bee to bee (qualitative and quantitative). Presumably, the contents of the tibial organs reflect the varied sources that the bees visit to collect chemicals, and probably also varies with the age and metabolic activity of the bee. Similar extracts of the thorax and abdomen contain only trace quantities of alkanes and alkenes. Table V presents the mandibular gland compounds and their distribution in a number of *Eulaema* and *Euglossa* species.

An example of the compositions of a floral fragrance and head and tibial extracts is shown in Figure 3. The figures are total ion chromatograms of the respective samples. Figure 3A shows a fragrance sample of *Gongora quinquenervis* from El Valle de Anton, Panama. At this site *G. quinquenervis* is avidly visited and pollinated only by *Euglossa deceptrix*. The fragrance is dominated by beta-ocimene and methyl-p-methoxycinnamate. Figure 3B shows a chromatogram of the hind tibia of a specimen of *E. deceptrix*. The individual bee was collected at *G. quinquenervis* flowers. The tibial extract contains methyl-p-methoxycinnamate, benzyl benzoate, several unidentified compounds, and a large amount of eicos-10-enyl-1, 20-diacetate. The head extract shown in Figure 3C contains none of the floral fragrance compounds, but it does contain large amounts of the diacetate.

The complexity of the extracts ranges from a single compound in *Euglossa sapphirina* to nearly twenty in *Eulaema cingulata*. Some of the compounds have not been completely identified; the position of double bonds is not known for many of the unsaturated compounds. We should soon complete the chemical determinations, and hope to extend the survey to include about 50 species.

Even in this limited sample, some taxonomically interesting patterns are present at the generic level. *Eulaema* secretions are complex, with large amounts of alkanes, alkenes, and acetates. *Euglossa* secretions are usually dominated by eicosenyl-1, 20-diacetate, with one or a few other compounds present. *Eufriesea* is not included in the table, but contain alkanes, alkenes, and a distinctive set of compounds not found in the other genera. *Euglossa intersecta* is morphologically atypical for the genus and resembles *Eufriesea* in a number of characters, but its mandibular glands contain the diacetate common to most *Euglossa*.

Euglossine species differ markedly in their preferences for various fragrance chemicals. Dressler (1982) listed the attractiveness of eight chemicals to 36 species of Panamanian *Euglossa*. Some bees are not attracted to any known baits; others are attracted to only a few (e.g. *Euglossa cyanura* to p-cresol), but most are strongly attracted to two or three compounds. Cineole appears to be the best known attractant (in terms of numbers of individuals and species), with methyl salicylate, skatole, vanillin, and eugenol also ranked highly. If there is a functional relationship between the fragrances that a bee species collects and the chemicals in its mandibular glands, then one might expect the presence of a given mandibular gland compound to be correlated with a preference for a certain chemical or set of chemicals in euglossines. From the available data, it seems that the mandibular gland contents are not a good



[illegible]

+ = a major compound.

\* = a minor compound, — = not present.



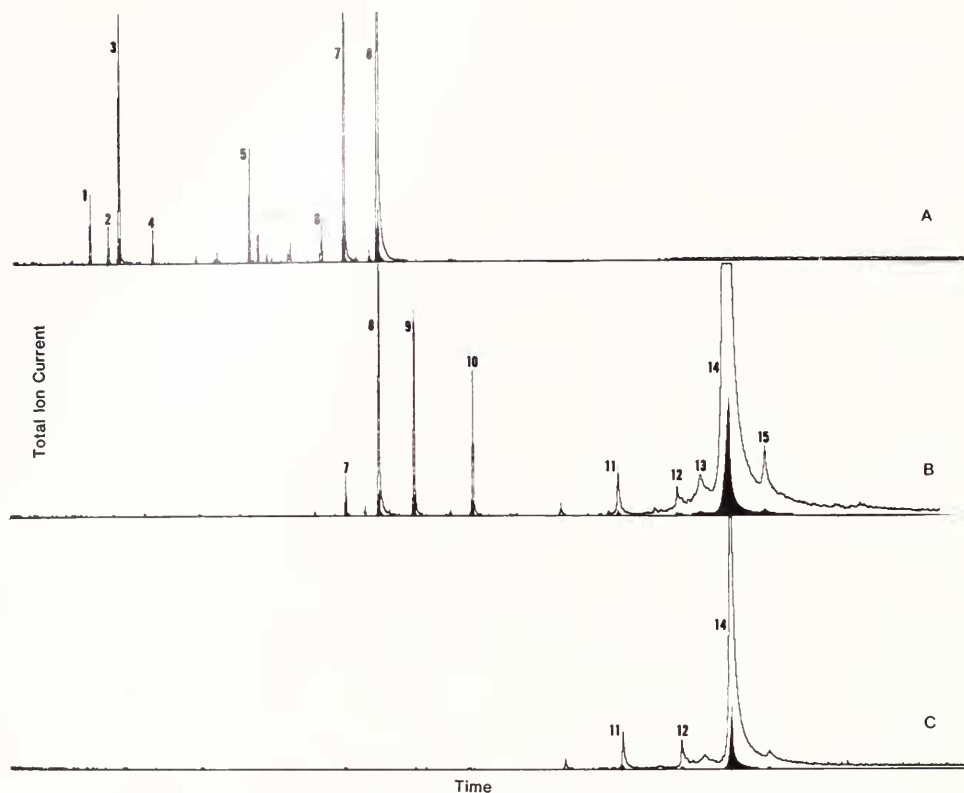


FIGURE 3, A-C. Total ion chromatograms of the floral fragrance of an orchid and extracts of the bee that pollinates it. A. Total ion chromatogram of the fragrance of *Gongora quinquenervis* from El Valle de Anton, Panama. B. Total ion chromatogram of hind tibial extract of *Euglossa deceptrix*, the pollinator of *G. quinquenervis* at El Valle. C. Total ion chromatogram of the cephalic extract of the same individual bee. Note the sets of compounds shared between A and B and between B and C. See text for details. Identification of peaks: 1 = beta-ocimene; 2 = terpinolene; 3 unidentified; 4 = methylphenylacetate; 5 = eugenol; 6 = methyl cinnamate; 7 = cis-methyl-p-methoxycinnamate; 8 = trans-methyl-p-methoxycinnamate; 9 = benzyl benzoate; 10 unidentified; 11 unidentified acetate; 12 unidentified; 13 unidentified; 14 = eicos-10-enyl-1,20-diacetate; 15 = n-nonacosene.

predictor of a species' fragrance preference. One possible exception is that the few *Euglossa* species which lack the eicosenyl diacetate are not attracted to cineole.

Figure 4 summarizes the distribution of these compounds within the male bee and diagrams our hypothesis of the fate of the floral fragrance compounds. We suspect that the fragrance compounds are absorbed into the tibial organ and are metabolized there to form the long-chain alkanes, alkenes, acetates, etc. These compounds would be transported via the hemolymph (possibly via sequestration) to the mandibular glands and stored in the reservoir. Obviously, experiments using radioactively-labeled fragrance compounds will be needed to test these hypotheses. The current data can only demonstrate that the mandibular glands and the tibial organs share a common set of compounds which are often species-specific, and it still seems reasonable to suspect that the collected fragrances serve as precursors for these large compounds.



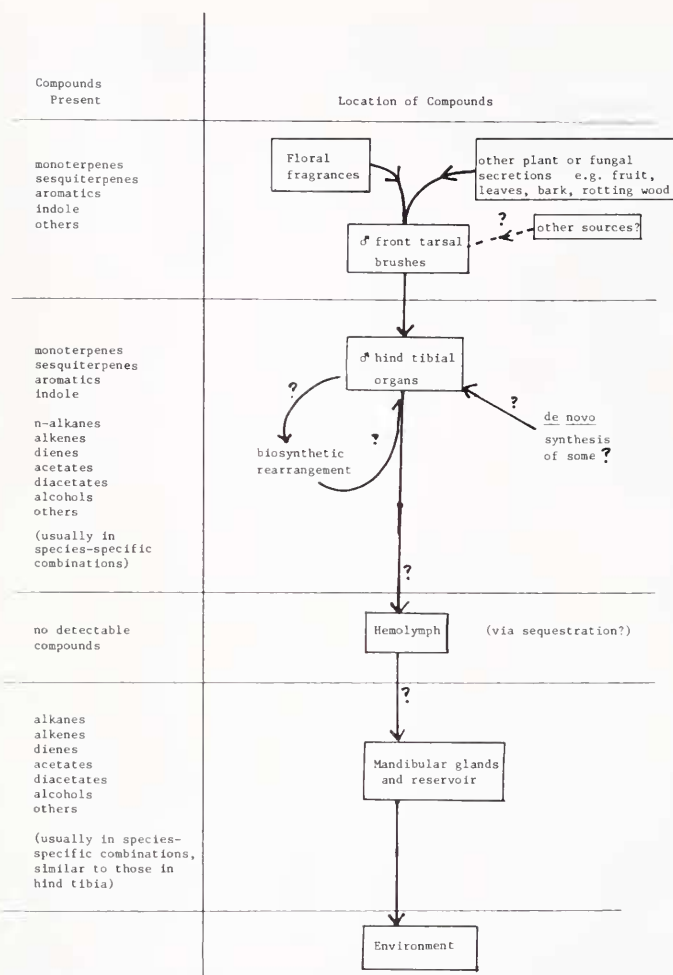


FIGURE 4. Hypothesized relationships between orchid floral fragrances and compounds occurring in hind tibial organs and mandibular glands of male euglossine bees. See text for discussion.

There have been a few reports of male *Eufriesea purpurata* brushing on surfaces treated with insecticidal sprays (van der Pijl and Dodson, 1966; Dressler, 1967; Roberts *et al.*, 1982). Roberts *et al.* reported that technical grade DDT, used for malaria control, is an excellent attractant of male *Eufriesea purpurata* in Amazonas, Brazil. Analysis of the body parts of the bees revealed astonishingly high concentrations of DDT, especially in the hind legs. It is not clear that DDT is the actual attractant of *Ef. purpurata* since pure DDT was not tested, but the results confirm that the bees can tolerate doses of DDT tens or hundreds of times greater than most insects. The report by Roberts *et al.* raises more questions than it answers, but it does suggest that the bees might be able to sequester or metabolize otherwise toxic doses of novel chemicals.

Since we still do not understand the role of the mandibular gland secretions in the euglossine life cycle, it is instructive to compare the available data on euglossines

with other related bees. Male bumblebees (*Bombus*) are known to mark territorial sites with the contents of their mandibular or labial glands. The secretions attract both males and females of the same species and are thought to play some role in their mating behavior (Kullenberg *et al.*, 1970). Interestingly, a number of compounds in *Bombus* and *Psithyrus* secretions are also found in male euglossines. These include primary alkanes, alkenes, alcohols, and acetates (Bergstrom *et al.*, 1968; Kullenberg *et al.*, 1970). Some *Bombus* also produce minor amounts of geraniol, citronellol, geranyl acetate, farnesol, geranylgeraniol, and geranylcitronellol. Such compounds have not been detected in euglossine mandibular glands, but they would not be out of place in the tibial organs. Observations of male euglossines performing territorial displays are not common, and sightings of courtship and mating are rare. Males of several species are known to establish territories centered around a tree trunk (often in a tree fall clearing), to patrol and defend the area against other conspecific males, and to display on perches and mate with females that enter the territory (Kimsey, 1980). A number of male territories may be aggregated in a favorable site, such as a large tree fall, but there is no evidence to suggest that males actively form leks. Kimsey also states that the males do not open their mandibles while perching and displaying, and sees no evidence that the males mark their territory with mandibular gland secretions. She also suggests that pheromones might be used only for short-range communication and mating behavior, not for a long-range attraction of females. The high molecular weight of many of the mandibular gland compounds would support this suggestion.

It is possible that the mandibular gland compounds are used for purposes other than territoriality or mating. Anyone who has collected and handled live male euglossines, especially the larger *Eulaema* and *Eufriesea*, often notices an odd, slightly rancid odor released from the bees as they are handled. The odor is similar to that of the mandibular gland contents, and it seems likely that the bees release mandibular gland secretions when disturbed. This suggests that the odor may represent a defensive secretion or alarm pheromone. When captured, the bees attempt to bite repeatedly, opening and closing the mandibles. Since the mandibular gland duct is thought to open and close with the movement of the mandibles, this may represent nothing more than an inadvertent release of the reservoir contents. After collecting a large number of male bees, the insect net sometimes becomes tainted with the odor, yet the net seems to have little effect on the wariness of other fragrance-collecting bees. It seems improbable that an alarm pheromone would consist of a complex, species-specific mixture of large molecules of low volatility, but the secretions may serve some role in defense against predators.

#### *Directions for future research*

It is clear that many of the facets of the orchid/euglossine interaction are not well understood, and there are numerous profitable areas for further research. Even the alpha taxonomy of the two groups is incomplete. Some suggestions for future research are listed below.

1. Perhaps the most critical need is to perform tracer experiments with  $^{14}\text{C}$ -labeled fragrance compounds. It is possible to maintain at least some species of euglossines in large flight cages for weeks or months (Ackerman, Kimsey; pers. comm.). Captive bees could be permitted to collect labeled fragrances and later sacrificed and examined for the presence and composition of labeled compounds in various body parts. We hope to attempt this in the near future with several of

the more common Panamanian *Eulaema* and *Euglossa*. The results of such experiments should help to direct subsequent studies of euglossine biology.

2. Assuming the mandibular gland compounds are functionally related to the fragrances, the chemical survey of head and tibial extracts should be extended to as many taxa as possible. Some euglossine species show interesting geographic variation in morphology and coloration; we do not know whether fragrance preferences and/or mandibular gland contents also vary geographically.

The complete characterization of mandibular gland contents should allow synthesis of individual components and allow us to field-test them singly and in combinations. This approach has been useful in studying similar problems in *Bombus* and other bees, and in the *Ophrys* pseudocopulation system.

3. We need to analyze the fragrances of as many androeglossophilous orchids, aroids, and other plants as possible. Some of the compounds appear to be novel, and many others present a great challenge to identify with samples of a milligram or less. We expect the analyses of fragrances and hind tibia to yield a number of new attractants for male euglossines. This should accelerate the collection of new or poorly known bee species. It would also be interesting to compare fragrances of euglossine-pollinated orchids with those pollinated by insects other than euglossines.

4. Much work remains in testing various fragrance compounds as attractants. Especially needed are experiments testing the attractiveness of various isomers (of known purity) of a given compound. There are little data on geographical and seasonal variation in the bees' response to baits, and on the attractiveness of pure compounds vs. mixtures.

5. Dressler (1976, 1982) discussed the utility of fragrance baits as a tool for studying orchid pollination. A small fraction (usually 5% or less) of bees caught at baits carry the pollinaria of various orchids. Many of the pollinaria can be identified to genus and sometimes species. Since a bee carrying a pollinarium is usually a legitimate pollinator of the orchid, a great deal of information can be obtained by baiting for bees and examining the pollinaria they carry. Occasionally, a bee may carry a pollinarium that cannot be associated with any known orchids and will spur a search for an undescribed orchid species (*Sievekingia*; Ackerman, pers. comm.).

6. The inter- and intrapopulational variation in fragrance composition should be examined for a variety of orchid species. Some genera may contain numerous, poorly differentiated fragrance forms, while others may possess consistent, species-specific fragrances. We are currently studying variation in fragrances and pollinators in the *Gongora* species of central Panama, and geographic variation appears to be great.

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